Circular No. 855

July 1950 • Washington, D. C.

UNITED STATES DEPARTMENT OF AGRICULTURE

Cinchona Root and Collar Rot in Peru and Bolivia[1]

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CONTENTS

Reawakening of interest in South American cinchona as a result of the loss of the East Indian sources of quinine and related alkaloids early in the course of World War II is now history. The work of locating native American stands of cinchona for exploitation and return to the Americas of seedling cinchona trees produced by the United States Department of Agriculture from seed collected just before the loss of the Philippines has been reported in a number of publications.

[1] A contribution from the Estacion Experimental Agricola en Tingo Maria, Peru, a technical agricultural service organization for the Oriente of Peru, operated jointly by the Direccion de Colonizacion y Asuntos Orientales, Ministry of Agriculture of Peru, and by the Office of Foreign Agricultural Relations, U. S. Department of Agriculture. This study was made possible by funds provided through the United States Interdepartmental Committee on Scientific and Cultural Cooperation and funds from the Government of Peru. The work herein reported is in cooperation with the Division of Forest Pathology, Bureau of Plant Industry, Soils, and Agricultural Engineering, U. S. Department of Agriculture, Beltsville, Md.

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Less known was the attempt to replace the exploited wild cinchona by introducing plantation culture of the species. In Bolivia this was accomplished by giving aid to private growers and in Peru by establishing an experimental plantation in cooperation with the Peruvian Government. In the establishment of this new forest crop, disease problems made their appearance. The United States Board of Economic Warfare, later the United States Foreign Economic Administration, requested technical assistance from the United States Department of Agriculture, and in 1943 the writer was assigned to the Cooperative Agricultural Experiment Station at Tingo Maria, Peru, to investigate, among other problems, the cinchona diseases.

The plantation problems in Bolivia, while of a serious nature, were found to be almost entirely of physiological origin (*8*, *16*) and are reviewed along with those of the Peruvian plantations primarily because of the insight obtained from them into the habits and requirements of the cinchona. The Peruvian plantation problems, by contrast, though having physiological aspects that predisposed the trees to attack (*16*), were due to the presence of a vigorous parasite (*5*, *8*).

HISTORY OF CINCHONA PLANTATIONS

In Bolivia attempts to produce cinchona in plantations predates the development of East Indian plantations. The plantations at Mapiri (fig. 1), dealt with herein, were started between 1938 and 1940. The planting stock used was exclusively the native *Cinchona officinalis* L., called locally *C. calisaya* Weddell. This is the species from which the Ledger type of *C. officinalis* L. (called *C. ledgeriana* Moens) was selected by the plant explorer Charles Ledger. The Bolivian cinchona type is characterized by a quinine content, expressed as the sulfate, of about 5 percent. Seeds are collected from wild forest trees and scattered on a piece of cleared ground at the site of the plantation to be established without use of a formal nursery. The seedlings that result are set about 1 year later directly in the plantation.

In Peru, except for a few small-scale attempts, plantation production of cinchona is of recent origin. Plantations were established at Hacienda Punizas (*14*) about 1927 and those at Fundo Sinchono, in which most of the work herein reported was done, in 1941 (fig. 1). Most of the planting stock is grown from seed produced on the trees of the original 1927 Japanese plantation (*14*). This stock was apparently obtained from Java and ranges in alkaloid content, expressed as anhydrous quinine, from less than 5 to 12.5 percent (*13*) (up to 16.7 percent quinine sulfate). The trees are produced in carefully managed nurseries and are sent to the plantation after one transplanting (1–1 stock). An additional plantation development in Peru, not concerned in this study, has been made in the Tambopata Valley (fig. 1) using native *C. officinalis* L. (*1*). No disease problems similar to those discussed herein have been reported at the latter plantation.

The plantations are located in mountainous regions within the natural range of *C. officinalis* L. (fig. 1). This region is characterized by annual rainfall of more than 100 inches, altitudes of from 5,000 to 8,000 feet, and by the presence of clouds and fogs that bathe the plantations for part of almost every day. Soils in the plantations, below a rather shallow humus and topsoil layer, are typically heavy clays.

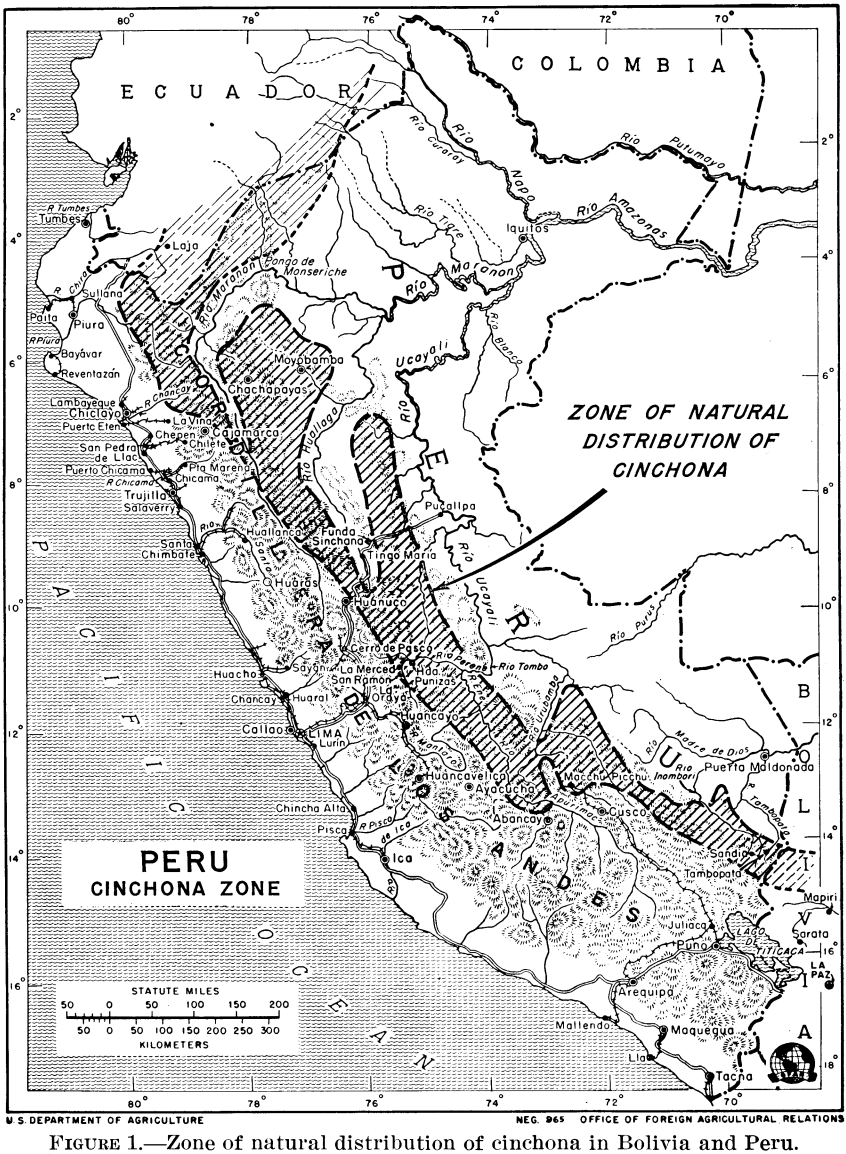

FIGURE 1.—Zone of natural distribution of cinchona in Bolivia and Peru.

HISTORY OF CINCHONA ROOT DISEASE

South and Central American literature on cinchona is quite scarce and other than a few recently published notes deals mainly with the culture of the species. In the East Indies and Asia, considering the recent introduction of the species, the literature available is amazingly abundant, and especially striking is the fact that much of it is on diseases,[2] particularly on root diseases. The pathogens blamed for

[2] A very complete review of cinchona diseases has been made by Lombard (*12*).

these root diseases are varied. Among those commonly mentioned are *Armillaria mellea* Vahl ex. Fr., causing a root canker disease; *Fomes noxius* Corner, causing brown root disease; *F. semitostus* Berk. and *F. lignosus* (Klotzsch) Bres., causing white root disease; *Rosellinia arcuata* Petch and *R. bunoides* (Berk. and Br.) Sacc., causing gray root rot; *Ganoderma pseudoferreum* (Wakef.) v. Over. and Steinm., causing red root rot; and *Sporedesmium* sp., *Fusarium* sp., and physiological root disease, occurring on cinchona on damp soils. The rather considerable number of different pathogens, all reported causing root disease, either indicates that cinchona as a species is rather susceptible to this type of trouble or that some further common denominator for the root diseases should be looked for. In general, Ledger type *C. officinalis* has been more susceptible to root disease than other species.

The more recent literature indicates that members of the genus *Phytophthora* are responsible for some part of the root troubles induced by pathogens. Coster (*4*) in 1941 reported an undesignated *Phytophthora* sp. associated with cinchona stripe canker in Java, and in 1940 Thompson (*17*) reported *P. cinnamomi* Rands causing cinchona root rot in British Malaya. Sawada (*15*) in 1936 reported a seedling blight that differed from other cinchona seedling blights in that the infection court was in the stem with the pathogen, *P. cinchonae* Saw., subsequently moving upward to kill the top. In Indochina (*2*) collar rot, thought to be caused by *Fusarium* sp. but with reported symptoms similar to those caused by *Phytophthora* spp., was so severe below 1,100 meters that trees were exploited between 5 and 10 years of age.

None of the *Phytophthora* species reported correspond to the species causing the root and collar rot of cinchona in Peru, although *P. cinnamomi* has recently been reported causing a nursery root rot at low altitudes (*6*). However, the abundance of reports of cinchona root diseases and the recent reports giving *Phytophthora* as the casual parasite indicate that members of the genus *Cinchona* are exacting in their requirements. They also indicate that when the conditions required for growth depart from the optimum to only a slight extent, parasites, such as Phytophthoras, which are generally favored by the very conditions that operate against the host, cause considerable losses. Under slightly more adverse site conditions normally saprophytic fungi appear to be the cause of heavy losses; whereas actually they are only the agent delivering the final blow to a host plant already dying. The probability also exists that many of the diseases reported could have been caused by members of the genus *Phytophthora*. This genus often is masked by the later invasion of saprophytes and weak parasites. This actually occurred in the Peruvian plantations and has been reported in connection with the "ink disease" of chestnuts in Europe caused by *P. cambivora* (Petri) Buis. and *P. cinnamomi* (*10*) and also with the chestnut root rot in the United States caused by *P. cinnamomi* (*9*). Reports in the files of the Office of Foreign Agricultural Relations and observations by the writer indicate that root diseases are a problem also in Guatemala, Costa Rica, and Ecuador; in other words, almost without exception wherever cinchona is grown in plantations in the Americas.

In addition to the reports of root troubles, *Phytophthora* has been reported causing wilts and dieback of cinchona in the nursery and

plantation. Celino (*3*)[3] in the Philippines and Kheswalla (*11*) in India reported seedling blights of cinchona caused by *P. palmivora* Butler and in the Western Hemisphere *P. parasitica* Dast. has been reported causing wilt and dieback in nurseries and plantations (*7*).

ROOT DISEASE IN THE BOLIVIAN PLANTATIONS

Symptoms

Through the third year, trees in Bolivia generally presented a thrifty appearance, but in the fourth year a decline set in, characterized by general slowing down of growth, reduction in the size and number of the leaves, and eventually loss of the normal green color of the leaves that remained. The leaves developed a reddish color and only toward the last showed signs of wilting. This final wilting and death usually occurred during the fifth year, and by the end of the sixth year most of the trees in the plantation were dead. To the observer the most striking feature of the problem was the very uniform appearance of the symptoms in the plantation. When various aged plantations were examined, the symptoms just described were observed in all stages, with plantations of 6 years and older presenting the appearance of millions of trees standing dead and gray-colored. The above-ground portions of the trees showed no evidence of any abnormal condition that could account for the symptoms. The root system, however, was another story. Because of too deep planting (fig. 2, *A*, *B*) the root system developed slowly and in general grew up parallel to the collar until it reached the topsoil and started to spread out. By the third year the ball of roots had enlarged to such an extent that girdling took place (fig. 2, *D*) and the tree progressively declined. In a very few cases, evidence of attack by fungi was found on some of the main roots and collars. After the third year many of the smaller feeding roots were dead. Some evidence of bacterial decomposition was found in the interior of the root ball. Trees showing advanced symptoms of trouble could be snapped off at the root ball.

Predisposing Conditions

Wild *Cinchona officinalis* growing in the vicinity of the plantations has its root system, with the exception of a limited taproot growth, located in the topsoil and humus layer of the forest floor. When the plantation site was prepared, the original forest cover was felled and left lying on the ground until it dried out thoroughly. The larger branches were bucked off and piled on the trunks. When the entire site was dry, this tremendous mass of wood, vines, and leaves was burned. Thus, not only was all vegetation destroyed but also the humus layer was burned, leaving only a shallow topsoil over the underlying clay. For reasons not too well understood by the plantation owners themselves, but apparently owing in part to local custom (*16*) and in part to advice given in the prewar period by buyers representing the quinine cartel, who were apparently not qualified agricultural experts on cinchona, the seedling cinchona trees were planted in the bottom of a hole about 2 feet deep (fig. 2, *A*, *B*). This hole was

[3] Published as *P. faberi* Maubl., a synonym of *P. palmivora* (*18*).

dug some days or weeks before the time of planting and was not backfilled in the sense of preparing a better site for the tree—rather, the tree was planted in a depression scooped in the bottom of the hole. During the first year the tree was entirely below the ground line and was usually shaded by fern fronds placed over the hole. During the wet season the hole remained full of water much of the time. The hole gradually filled up with leaf litter and soil, and the roots grew upward until they were able to enter the looser topsoil and make normal radial growth. By the third or fourth year there was still a pronounced depression around each tree. It seems evident that the actual cause of the death of the trees was the girdling action that resulted when

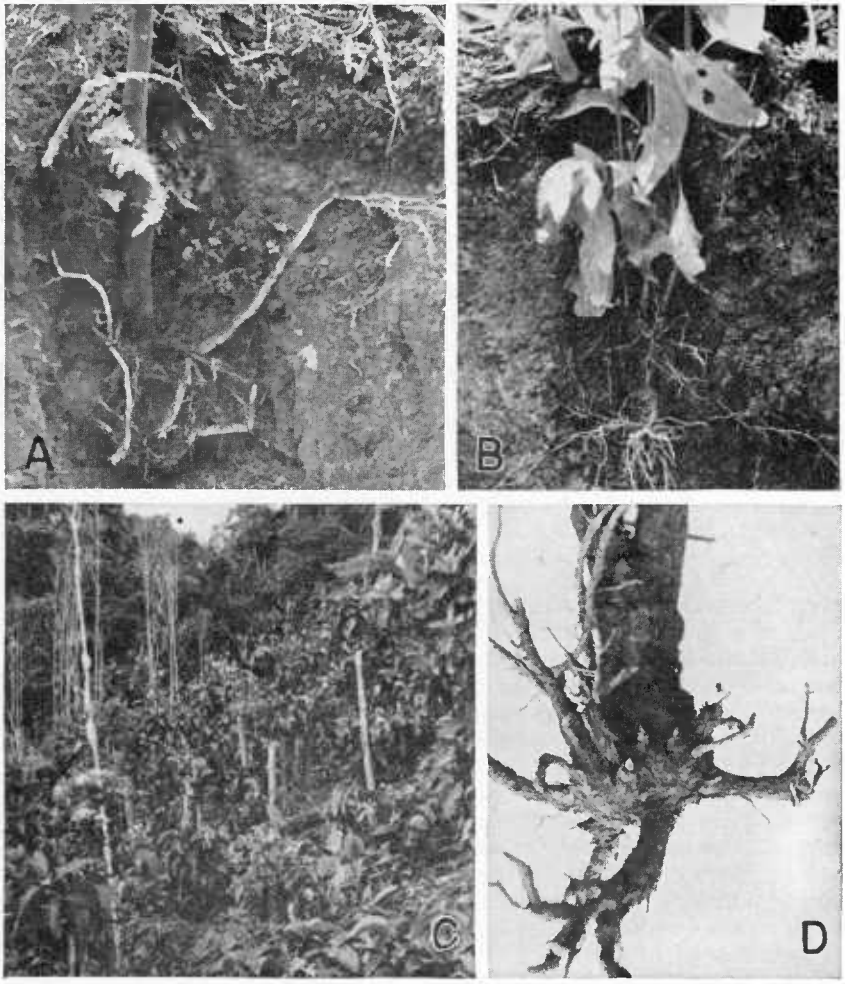

FIGURE 2.—*A*. Three-year-old *C. officinalis* showing growth under the deep-hole planting system in use in Bolivia. *B*. One-and-one-half-year-old cinchona planted by same system. *C*. Regeneration by sprouts in Bolivian plantation from 5-year-old cinchona that had died back as a result of deep-hole planting. *D*. Appearance of the root system of a 4-year-old cinchona dying as a result of deep-hole planting in Bolivian plantation.

growth caused the radial enlargement of the doubled-up roots (fig. 2, *D*). This condition may have been aggravated to some extent by action of normally saprophytic fungi and bacteria but, considering the very favorable conditions present for such attack, it seems that these played a very minor role and that the direct cause of the loss was the starvation of the roots, due to girdling. Further evidence of the nonparasitic nature of the Bolivian plantation losses exists in the considerable number of trees that survived and eventually grew from suckers developing from a portion of the root system (fig. 2, *C*).

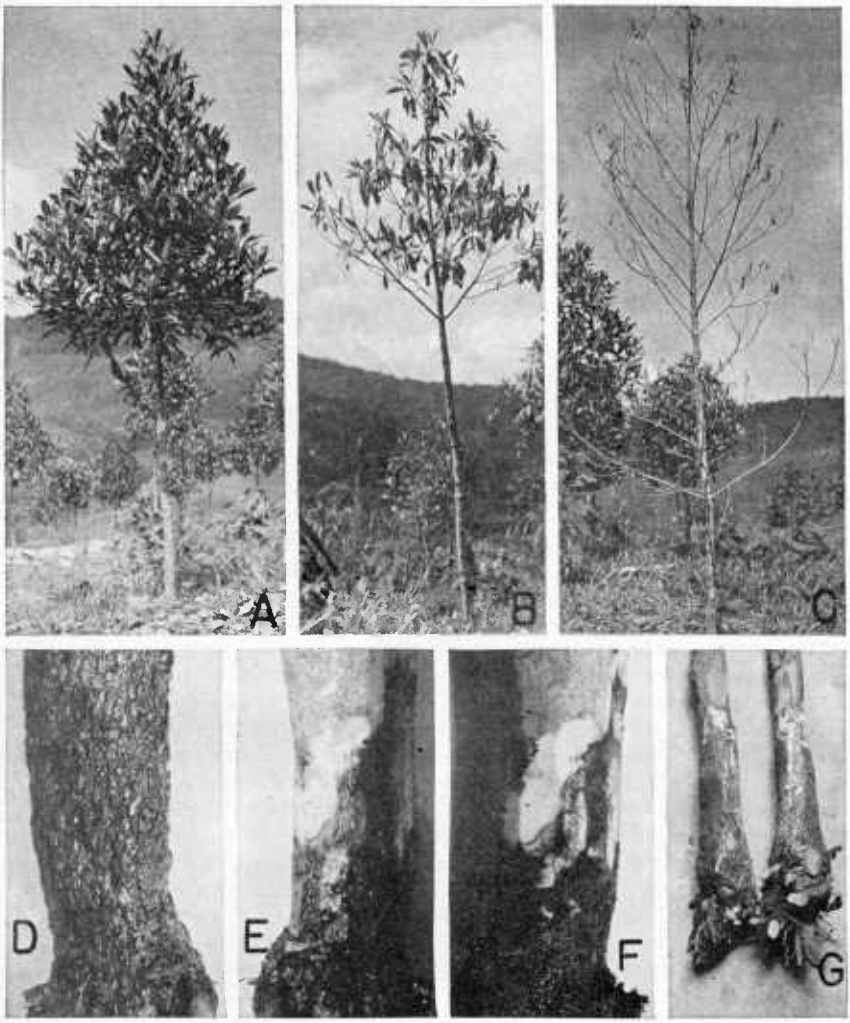

FIGURE 3.—*A*. Healthy-appearing 3-year-old Peruvian Ledger type *C. officinalis*. *B* and *C*. Progressive decline due to root and collar rot caused by *Phytophthora quininea*. *D*. Swelling in collar region, indicating girdling caused by collar infection. *E*. *P. quininea* infection in phloem tissue. *F*. Same section as *E*, cut to the cambium tissue to show advancing edges of infection. *G*. White mycelial fans of *Armillaria mellea* present between bark and wood in dying cinchona in Peruvian plantation.

ROOT DISEASE IN THE PERUVIAN PLANTATIONS

Symptoms

In Peru the plantation trees of the Ledger type *C. officiralis* were affected in all ages, but heaviest losses occurred just after planting and again at about the third year. After this age, losses apparently decreased. On the basis of limited observations, it appears that there is an increase in the incidence of disease after the eighth year, when the growth rate of the cinchona slows down (*13*). In the nursery, which contains trees under one year of age, the disease attacks trees mainly of transplanting age and is seldom found on seedlings.

Above-ground signs of the disease, in both nursery- and plantation-age trees, are, in the early stages, slight chlorosis of the leaves, followed in later stages by loss of the chlorophyll and appearance of a reddish color in the leaves. Eventually most of the leaves wilt and fall, so that only small, reddish-colored, terminal leaves remain on the tree as it approaches death (fig. 3, *A*, *B*, *C*). A pronounced swelling (fig. 3, *D*) just above the ground line, indicating girdling, may be present or absent but is usually present.

Coinciding with these above-ground signs, the internal symptom is decay of all or part of the collar region extending into the stem as high as 8 inches above the ground and into the basal portions of the connecting roots. The entire root system or part of the root system may be retted, and localized infections, unconnected with the main center of decay, may occur on the larger roots. In general, however, the disease is one that typically involves the collar region and the basal portions of the larger roots (fig. 3, *D*, *E*, *F*). The diseased root and collar tissue is dark brown in color. The advancing edge of the infection (fig. 3, *E*, *F*), in the cambial tissue and in the phloem, is cinnamon-red in color with the pathogen advancing into healthy tissue in wedge-shaped streaks. In a large part of the plantation trees examined, cream-colored mycelial fans were present (fig. 3, *G*) in the decayed tissue between the bark and the wood. In many cases these fans were present within a millimeter or two of the advancing edge of the infection. Rhizomorphs, proved by culture to be from the same fungus, were present in and on some of the older decayed tissue. Other Basidiomycetes, *Diplodia* and *Fusarium*, were occasionally found in the decayed tissue but their occurrence was of such limited extent that they obviously do not enter into the decay problem. The Basidiomycete, with cream-colored fans, occurs only rarely in nursery-age trees dying of root disease. Otherwise, external signs and internal symptoms are the same as in the plantation. In some nursery-age trees the disease involves the entire stem of both Ledger type *C. officinalis* and *C. pubescens* Vahl [4] seedlings and, in the absence of cultural diagnosis, can be confused with top wilt (*19*).

Limited observations of the signs and symptoms of this disease in both nursery- and plantation-age *C. pubescens* and *C. micrantha* Ruiz & Pavon trees show no marked differences from the appearance of the disease in Ledger type *C. officinalis*. However, an additional manifestation of the disease occurs. Above the normal infection in the collar region vertical streaks of infection are often found extending 10 to 20 centimeters up the stem. These streaks are often only 1 or

[4] In Guatemala called *G. succirubra* Pavon.

2 millimeters wide. This is especially significant when it is recalled that *C. pubescens* is the preferred rootstock for Ledger type cinchona throughout most of the cinchona-growing areas of the world in areas where root diseases are troublesome.

THE PATHOGEN

Isolation

Early in 1944, toward the end of the wet season, the first attempts were made to isolate the pathogen in pure culture. All isolations attempted were from the advancing margin of infection in roots or collar tissue of dying trees. With malt agar and standard cultural techniques, 20 percent of the isolations yielded the Basidiomycete producing the white mycelial fans. This fungus was isolated only from trees that had visible mycelial fans. An additional 20 percent of the isolations yielded *Trichoderma* sp., 50 percent yielded other Basidiomycetes, *Fusarium*- and *Diplodia*-like fungi, and 10 percent were negative. Fruiting bodies of the fungus producing the white mycelial fans have never been found in direct association with the fungus in cinchona. In culture, however, the fungus is indistinguishable from other cultures of *Armillaria mellea* and has been found fruiting in the plantations; hereafter it will be referred to as *A. mellea*.

Special methods for *Phytophthora* isolation were also used. These primarily consisted of placing sections of diseased root and collar tissue, containing only the advancing edge of the rot and a small portion of adjacent healthy tissue, in test tubes containing sterile water. Sections were kept in water for 3 days, with several changes of water being made, and were then lightly surface sterilized and plated on corn meal or prune agar. Typical growth of *Phytophthora* could be identified in 2 or 3 days. When these methods were used 12 percent of the cultures yielded *Phytophthora* sp., 12 percent *Trichoderma* sp., 4 percent other miscellaneous fungi but not *A. mellea*, and 70 percent were negative.

In subsequent attempts at isolation the percentage of *Phytophthora* sp. increased to about 15 percent of the total plates but in plantation trees never went above this amount and did not appear at all during the dry season. This, however, is not unusual for this genus. In the United States isolations of *P. cinnamomi* Rands from chestnut root rot never averaged better than 4 percent (*9*). The presence of *Trichoderma* sp. in many isolations may be significant as it has been reported as a parasite of *Phytophthora* (*9*, *19*) and the locally isolated strains were able to parasitize the cinchona root rot *Phytophthora* sp. in culture.

Cultures from diseased tissue of nursery-age cinchona, by standard methods, occasionally yielded the *Phytophthora* sp. but most of them were negative. When sections of the stem and collar containing actively invaded tissue were held in water and then cultured, almost 100 percent of the isolates were pure cultures of the *Phytophthora* sp. With the exception of an occasional isolate of *P. cinnamomi* from plants brought from nurseries in the lower altitude, where a different root disease occurred (*6*), the *Phytophthora* sp. isolated was the same as that obtained from plantation trees. *P. cinnamomi* has never been isolated from cases of root and collar rot in the plantation.

Identification

The *Phytophthora* sp. isolated from cinchona root and collar rot is a member of the group containing *P. cinnamomi*, *P. cambivora* (Petri) Buis., *P. erythroseptica* Pethyb., *P. cryptogea* Pethyb. & Laff., *P. richardiae* Buis., *P. drechsleri* Tucker, *P. megasperma* Drechsl., and *P. fragariae* Hickman, which are responsible for root diseases of many genera of plants. It is morphologically closely related to *P. cinnamomi* and *P. cambivora* but is distinct from all in this group and has been described as a new species, *P. quininea* Crandall (*5*). It may readily be distinguished from *P. cinnamomi*, which also is found on cinchona, by the absence of the grapelike clusters of chlamydospore-like bodies. *P. quininea* is characterized by the abundant production of large (45–90 μ), dark brown spherical chlamydospores after 132 hours in culture; by non-papillate, ovate to obpyriform, sporangia, 45.9–67.5 x 25.5–37.5 μ, which are produced only in running water; by subspherical to spherical oogonia, 67.5–82.5 μ, with paragynous antheridia; and by production of irregularly shaped vesicles that give a thickened appearance to the hyphae and cause irregular growth in culture.

Inoculations and Proof of Pathogenicity

The first trial of pathogenicity of *P. quininea* was made in late July 1944, with 500 1-year-old Ledger form *C. officinalis* transplants. One-half of the plants were located on a poorly drained site and the other half on a well-drained one. Plants in each location were inoculated in approximately equal numbers with three isolates of *P. quininea* from the plantation and one from the nursery, and, for comparison, an isolate of *P. cinnamomi* from the nursery and an isolate of *Armillaria mellea* from the plantation. An equal-sized group was used as control by inoculating with sterile agar. All plants were inoculated by cutting through the bark to the cambium phloem region just above the soil line, placing a millimeter-square block of oatmeal agar containing the fungus mycelium in contact with the wound, and covering it with cotton. First losses occurred at the end of 16 days and by the forty-second day all plants that had become infected had died. Re-isolations were made as the plants died and the inoculated fungus was recovered from the infected tissue that had typical symptoms of the disease as it appeared in nature. In all cases where infection occurred, the fungus spread into the below-ground collar region as well as upward into the stem. Only in the case of the *P. cinnamomi* isolate was there any difference between performance on the wet and dry site. This isolate was more virulent than the *P. quininea* isolates and killed 80 percent of the plants inoculated with it on the wet site and 67 percent of those on the dry site. The isolates of *P. quininea* killed 67, 53, 27, and 20 percent of the trees on the dry site and 67, 60, 40, and 33 percent of the trees on the wet site. None of the inoculations with *A. mellea* were successful and the checks likewise remained healthy.

First inoculation trials were made in the above-ground portion of the stem, and thus did not give the same opportunity for infection that occurred in nature. A second inoculation test was run in early August with the same number of plants, with wet and dry sites, but with the inoculations made below the soil line at the collar. First dying oc-

curred at the end of the 10th day and all infected plants were dead by the 26th day. *P. cinnamomi* killed 100 percent of the plants inoculated on the wet site and 63 percent of those inoculated on the dry site. Six isolates of *P. quininea*, 3 from the nursery, and 3 from the plantation, were used. On the wet site 5 isolates each killed 100 percent of the trees inoculated and 1 killed 83 percent. On the dry site 2 isolates each killed 100 percent, 3 killed 87 percent, and 1 killed 63 percent. Again in this test *A. mellea* caused no infections and all controls remained healthy.

Subsequently a field inoculation trial, with vigorously growing, healthy 3-year-old plantation trees, was set up with inoculations of *P. quininea* and *A. mellea* alone and in combinations. Results were inconclusive when an accident occurred 6 months later, but, at the time final notes were taken, a number of the trees inoculated with *P. quininea* were showing symptoms of the disease, a few of the combination inoculations showed growth of *A. mellea*, and none of *A. mellea* alone had resulted in infections. A smaller inoculation trial on 5-year-old trees was made with *A. mellea* at the older cinchona plantation at Hacienda Punizas. Fifteen trees inoculated in May 1944 were still healthy in appearance in late 1946.

Starting in September 1944 and continuing through July 1947, all available strains and species of *Cinchona* ranging in age from 1 to 3 years were inoculated in the search for a resistant type, either for use as a root stock or if suitable for clonal propagation for direct planting. Some 6,000 individuals, of which about half were of the Ledger form *C. officinalis*, were inoculated. This form has been found to be more resistant than the other *Cinchona* species tested and some 58 individuals did not become infected even after 3 separate inoculations. Only a few individuals of the local *C. officinalis*, *C. micrantha*, and *C. pubescens* remain of the hundreds inoculated, and no survivals remain of the *C. officinalis* [5] form from southern Peru or Bolivia.

During the 1944 inoculation trials 1-year-old transplants of *Hevea brasiliensis* (H. B. K.) Muell. Arg., *H. guianesis* Aubl. var. *lutea* (Spruce ex. Benth.) Ducke & Schultes, *Persea americana* Mill., *Ananas comosus* (L.) Merill, *Annona* sp., *Citrus aurantium* L., *Psidium guajava* L. and *Ladenbergia magnifolia* Kl. were inoculated. None of these species were susceptible to *P. quininea.*

Temperature Requirements of Phytophthora quininea

Two isolates of *Phytophthora quininea* on corn-meal agar (at the optimum pH of 6.0) were grown for 48 hours at 5°, 10°, 15°, 20°, 25°, 30°, 35°, and 40° C. No growth occurred at 5° and 10° C.; at 15° the isolates grew 2.3 and 5 millimeters; at 20° they grew 2.7 and 11 millimeters; at 25° (optimum), 8 and 16 millimeters; at 30°, 5 and 12.5 millimeters; and at 35° and 40° no growth occurred. Two isolates were selected for the test, one the fastest growing and one the slowest of those isolated. The isolates were not killed by the 5°, 10°, or 35° temperatures and they started growth after removal from the temperature chambers. Both isolates were from Fundo Sinchono, where the highest temperature recorded is 24.2° and the lowest 14.7° C. It thus appears that *P. quininea* would not be expected to be a

[5] Called *C. calisaya* Wedd. in southern Peru and Bolivia.

problem at much higher altitudes in Peru but could well be a serious factor at plantations at altitudes lower than 5,000 feet (Fundo Sinchono).

pH Requirements of Phytophthora quininea

Two types of isolates of *Phytophthora quininea* can be distinguished. These two types are identical for all regularly recorded morphological characters but differ in their growth rate (at optimum temperature of 25° C., 4 and 8 millimeters per 24 hours) because of the relative abundance of vesicle production. Both types make almost no growth at pH 8.5 or below 5.5. At pH 5.5 or 7.0, growth for both types is very irregular, due to the abundance of vesicles. At pH 6.0, growth is very regular for most isolates. A few have more regular growth at just above pH 6.0. Apparently the optimum pH is therefore around pH 6.0 but with growth possible from below 5.5 to 8.5.

EPIDEMIOLOGY

Spread From Nursery to Plantation

During the early part of the first studies of the cinchona root disease it was observed that no attempt was made at the nursery to cull infected or otherwise injured or retarded transplants. Further living seedlings from areas in the nursery that had suffered losses from root rot were sent to the plantation. This, together with the fact that before this time transplants were carried from the nursery to the plantation with a ball of soil, suggested that the disease could be of nursery origin. It has not been possible to demonstrate that the disease does not exist in plantation soils but it has been possible, by marking trees in the nursery, to demonstrate that infected trees invariably succumb to root disease after transplanting and, further, that transplants coming from areas in the nursery where root disease was prevalent often die later from the disease. Mapping of areas of dead and dying trees in the plantation, while showing more closely the correlation with site conditions, nonetheless gives evidence that infected areas center around foci of infections of earlier losses. In practice a considerable decrease in incidence of disease in the first year after planting resulted from severe culling in the nursery and transporting the plants to the field with their roots bare. As might be surmised, the reduction in costs for this part of the operation was even more pronounced than the decrease in disease incidence.

Effect of Soil pH on Incidence of Disease

Considerable variation in pH in the soils of the plantations and nurseries was observed. In the nurseries, where soil pH ranged from 4.2 to 6.8, it was possible to find areas of total loss and closely adjacent healthy areas for comparison. Sixteen areas containing healthy seedlings had pH readings ranging between pH 4.2 and 6.4. Sixteen areas of 100-percent mortality had pH readings ranging from 4.2 to 6.8. Less variation was found in the plantation on any given area although pH ranged from 4.0 to 6.5. Diseased trees found on a soil of any given pH were adjacent to healthy trees on soils of the same pH. It

was therefore concluded that, with the exception of heavier clay soils that had low pH and where the disease was more severe for other reasons, there was no relation between soil pH and disease incidence.

Conditions Predisposing to Attack

Field observations and experiments showed that four factors, under local conditions inseparable, were the basis of conditions that opened the way for attack by *Phytophthora quininea*. These four factors are soil type, slope, drainage, and planting methods, with the last, at the time work on the disease was initiated, being in a sense a product of the other three. At the time the plantations were started, a planting method using a deep hole or hueco (*16*), not as pronounced as that used in Bolivia, was adapted for use on cinchona from experience on other tree crops in the zone. Actually this hueco planting may be the result of the wasteful land-clearing methods, which result in cutting and burning the tree cover along with whatever organic topsoil exists, thus encouraging the use of the hueco on steep slopes for lack of loose soil in which to plant. Basically the method in use consisted of digging a rectangular hole about 12 x 12 x 36 inches some weeks or even months before planting time and backfilling this hole with topsoil. At the time of planting, a hole of sufficient size to accommodate the ball of earth on the cinchona tree was scooped in the then depressed surface of the fill. The tree with its earth ball was then forced by hand into the hole as far as it would go, usually carrying the collar 3 to 4 inches below the soil line. Topsoil was then scraped into the hole to make it level with the surrounding soil. Subsequently rains caused the soil in the hole to settle 4 or 5 inches more, with the final result that not only was the original collar line of the tree often 6 inches below the soil line but, in addition, the soil line in the hole was anywhere from a slight depression to 6 inches below the surrounding undisturbed soil. On the steep slopes, which in the cinchona plantations range from 35 to 70 degrees and are usually of heavy clay soils, these holes are cut even deeper so that drainage was almost impossible. On flat sites or in depressions the holes were full of water for almost half the year. Only on the gentle slopes and tops of ridges where the soil is loose and friable did the hole and its adjacent soil eventually merge and even here the collar of the tree was often 6 inches below the ground line, although not in a depression.

The above-described conditions are ideal for the growth and attack on a host by a member of the genus *Phytophthora* as well as very unfavorable for growth of cinchona with or without the presence of a parasite. Many of the trees examined, which were dying of root disease on very unfavorable sites and from which *Armillaria mellea* only was isolated, could have been dying from the effects of site and *Armillaria* alone without the added attack of the *Phytophthora*. Data taken in 1944 on rows of 3–1 plantation trees show clearly the effects of site in combination with the hueco planting method. On the lower, gentle slopes no losses had yet occurred; higher up, as the slopes became steeper, losses of 20 percent had occurred; on the first bench the losses were still 20 percent; higher up, as the heavier clays became more in evidence, losses reached 50 percent; the next bench, with heavy clay soil, had a 95 percent loss; and above this bench the steep clay slopes continued with losses between 50 and 60 percent. Total losses

in rows planted on this type of site (typical of the plantations) ranged between 47 and 75 percent.

An experiment was started during the 1943 planting season, with 8 rows of trees on a steep clay slope. Each row, depending on the terrain, contained between 36 and 43 trees. Alternate rows were planted by the previously described method then in use (hueco-planted), but with some care to avoid planting the tree too deeply, and the adjacent row planted by preparing a small hole with a mattock at the time of planting and setting the tree so that it was on a slight mound with the original collar at the soil line. Unculled trees, as then sent from the nursery with an earth ball, were used, with no attempt at selection. When last examined early in 1947 (4 years later), losses in the hueco-planted rows averaged 56 percent and in the mattock-planted trees 29 percent.

Also in 1943, areas of total loss on well-drained sites were replanted as carefully as possible. The replants on the poorly drained sites suffered a 100 percent mortality from Phytophthora root rot within a few months, while those on the well-drained sites have almost all survived.

In the nursery, losses are always severe on poorly drained and low areas in the transplant beds.

Evidence on the Origin of Phytophthora quininea

It has thus far been impossible to secure any positive proof that *Phytophthora quininea*, the pathogen causing Cinchona root rot, is an introduced fungus. *P. quininea* thus far has been found only in the plantations based on introduced Ledger form *C. officinalis* and has not been found in nature on *C. officinalis* or other species of *Cinchona*, either adjacent to the plantations where the disease occurs or in other parts of the cinchona range. Predisposing conditions, ideal for development of the disease, are found in Bolivia but the disease was not observed there. The local *C. officinalis* and the *C. officinalis* of southern Peru and of Bolivia have been shown by inoculation to be more susceptible to *P. quininea* than the introduced Ledger form selections. Local selections of *C. micrantha* and *C. pubescens* introduced from Guatemala are equally highly susceptible. In the East Indies and in Central America *C. pubescens* is generally considered to be resistant to root diseases and is used as a root stock on which to graft the more susceptible Ledger selection.

The first observations in 1943 indicated that the disease was being carried from the nurseries to the plantation. The fact that the optimum temperature for growth of *P. quininea* and the temperature range are almost the same as the temperature of the cinchona plantation where the disease was first found is not conclusive since the plantation was intentionally located at a site where altitude, temperature, and rainfall were close to the optimum for Ledger form cinchona, as reported from the East Indies. The conflicting evidence thus apparently allows either of two surmises to be made: (1) That *P. quininea* has been introduced into the zone of the cinchona plantations where it occurs, but from where or from what host remaining as unanswered questions, or (2) that under natural conditions for cinchona in the forest association the disease either is not a factor or is held in control by other soil organisms.

CONTROL

While the losses in Bolivia and in Peru are the result of different causes, one physiological and the other pathological, in the last analysis they stem from the same predisposing factor, the planting method. In practice the control measures recommended have been the same.

In Bolivia it was recommended that formal nurseries be established to produce hardy planting stock and that land-clearing methods be modified to avoid the destruction of the topsoil and thus permit the abandoning of the hueco planting method. It has not been possible to determine what practices are now in use in Bolivia or what has been the result of them.

In Peru the first changes recommended and carried out were to level and transplant beds, cull the transplants, and remove the soil from them before sending them to the field. Later, other recommendations were followed as far as possible: To pile the logs and branches from the original cover into convenient gullies or to burn them in selected locations, instead of burning them on the planting sites; to plant only on gentle slopes and the tops of ridges, where good friable soil occurs; and to abandon the hueco method of planting and to plant the trees with the root system in the topsoil layers, with a slight mound around each tree.

Study plots of about one-half acre each have been laid off in each of the following parts of the plantation: (1) Where, in 1943, the hueco method of planting had been used; (2) where, in 1944, the first bare-rooted and selected transplants had been set, with some modification in the planting method, principally in the nursery; and (3) where, in 1945, the trees had been planted according to full recommendations as finally recommended. Losses in plots from the first part are now 75 percent; from the second, 24 percent; and from the third, just under 20 percent. Of the 20-percent loss in the last-named area, 15 percent occurred at or soon after planting. Selections made for disease resistance show promise, but until they are clonally propagated and proved under all conditions they cannot be considered to be positive in their resistance.

SUMMARY

A root disease of the Ledger form of *Cinchona officinalis* L., caused by *Phytophthora quininea* Crandall, was found in two plantations and their nurseries located in the upper Ucayali River Basin of Peru. Attack by the pathogen in the plantation was the result of a predisposing condition provided by a deep-hole planting method. A somewhat similar planting method in use near Mapiri, Bolivia, resulted in heavy losses in plantations of *C. officinalis* L. from entirely physiological causes. In Peru, in addition to the nursery and plantation host originally found attacked, it has been demonstrated by artificial inoculation that Peruvian and Bolivian *C. officinalis*, *C. micrantha* Ruiz & Pavon, and *C. pubescens* Vahl are highly susceptible to attack by *P. quininea*. Control of the disease has been moderately successful by modifying nursery practices and plantation planting methods so as not to favor the pathogen and to favor the host. Selection of varieties of Ledger form cinchona resistant to the disease offer promise but need further testing.

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U. S. GOVERNMENT PRINTING OFFICE: 1950

For sale by the Superintendent of Documents, U. S. Government Printing Office
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